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Antiprotozoal and Antimicrobial Compounds from the Plant Pathogen *Septoria pistaciarum*

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Abstract

Four new 1,4-dihydroxy-5-phenyl-2-pyridinone alkaloids, 17-hydroxy-*N*-(*O*-methyl)septoriamycin A (**1**), 17-acetoxy-*N*-(*O*-methyl)septoriamycin A (**2**), 13-(*S*)-hydroxy-*N*-(*O*-methyl)septoriamycin A (**3**) and 13-(*R*)-hydroxy-*N*-(*O*-methyl)septoriamycin A (**4**), together with the known compounds (+)-cercosporin (**5**), (+)-14-*O*-acetylcercosporin (**6**), and (+)-di-*O*-acetylcercosporin (**7**), lumichrome, and brassicasterol were isolated from an ethyl acetate extract of a culture medium of *Septoria pistaciarum*. Methylation of septoriamycin A (**8**) with diazomethane yielded three di-*O*-methyl analogues, two of which existed as mixtures of rotamers. We previously reported antimalarial activity of septoriamycin A. This compound also exhibited significant activity against *Leishmania donovani* promastigotes. Compounds **5–7** showed moderate in vitro activity against *L. donovani* promastigotes and chloroquine-sensitive (D6) and -resistant (W2) strains of *Plasmodium falciparum*, whereas compound **5** was fairly active against methicillin-sensitive and methicillin-resistant strains of *Staphylococcus aureus*. Compounds **5–7** also displayed moderate phytotoxic activity against both a dicot (lettuce, *Lactuca sativa*) and a monocot (bentgrass, *Agrostis stolonifera*), and cytotoxicity against a panel of cell lines.

Phytotoxins from plant pathogenic fungi may act as inhibitors of plant-like metabolic pathways in the apicoplast, a chloroplast-like organelle, essential for the survival of plasmodium species.¹ Previously we reported² the isolation and identification of the 2-pyridinone alkaloid, septoriamycin A³ (**8**) and three of its derivatives from the causative agent of septoria leaf spot disease in pistachio, *Septoria pistaciarum* (Ascomycetes), as part of our program to identify antimalarial compounds from plant pathogenic fungi. Although we detected several minor alkaloids of the same class in the original extract, their structural elucidation was not possible due to insufficient quantities. In order to isolate the minor compounds, we recultured the same fungus under the identical conditions at a larger scale. In the ethyl acetate extract of this fermentation culture broth, a series of pigments were detected with 2-pyridinone alkaloidal architecture. The extract showed herbicidal, antimicrobial, antiplasmodial, and antileishmanial activities albeit with low selectivity indices. From this extract, four more new minor septoriamycin A analogues, 17-hydroxy-*N*-(*O*-methyl)septoriamycin A (**1**), 17-acetoxy-*N*-(*O*-methyl)septoriamycin A (**2**), 13-(*S*-

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hydroxy-*N*-(*O*-methyl)septoriamycin A (**3**), and 13-(*R*)-hydroxy-*N*-(*O*-methyl)septoriamycin A (**4**), in addition to the parent and its previously reported analogues were identified. Septoriamycin A (**8**), the major 2-pyridinone alkaloid in this extract showed significant antileishmanial activity in addition to its reported antiplasmodial activity. The pigments were identified as three known perylenequinones, (+)-cercosporin (**5**),⁴ (+)-14-*O*-acetylcercosporin (**6**),⁴ and (+)-di-*O*-acetylcercosporin (**7**).⁵ These pigments have previously been identified as phytotoxins produced by a number of phytopathogenic *Cercospora* species and have been linked to their pathogenicity.⁶ Their biosynthesis appeared to be controlled by numerous environmental and physiological factors and the presence of even small amounts of certain compounds in the medium was found to have a strong stimulatory or inhibitory effect on their production.^{6,7} Their ability to generate reactive oxygen species in the presence of light has been attributed to their phytotoxic activity.⁶ Cercosporin and its esters have also been reported to have antibacterial and antifungal⁸ activities as well as growth inhibitory effects on lettuce⁴ and tomato seeds.⁸ In this study, the perylenequinones showed antileishmanial, antiplasmodial, and cytotoxic activities in addition to antibacterial and antifungal activities. Two more known compounds, lumichrome,⁹ and brassicasterol¹⁰ were also isolated and identified.

RESULTS AND DISCUSSION

Fractionation of an EtOAc extract of a culture medium of *S. pistaciarum* by Sephadex LH-20 gel column chromatography followed by purification using silica gel and RP C₁₈ chromatography afforded four minor 2-pyridinone alkaloids **1–4** in addition to the known septoriamycin A (**8**) and its three derivatives,² three known perylenequinones (+)-cercosporin (**5**), (+)-14-*O*-acetylcercosporin (**6**), (+)-di-*O*-acetyl-cercosporin (**7**), lumichrome, and brassicasterol.

The molecular formula of compound **1** was determined as C₂₃H₃₁NO₅ by HRESIMS. UV maxima (205.0, 240.9, and 297.0 nm) and IR absorptions (3400, 3207, 1647, and 1555 cm⁻¹) of **1** were consistent with those of a 2-pyridinone moiety.² The aromatic regions of the ¹H NMR and COSY spectra were similar to those observed for 5-phenyl-2-pyridinones previously isolated from this species, and consisted of a one-proton singlet and an A₂B₂C spin system due to a monosubstituted phenyl group.² The aliphatic region exhibited two oxymethine and two methyl doublets indicating the presence of a 2,4-dimethyltetrahydropyran moiety and an *N*-methoxy group. HMBC correlations of H-2' and H-6' (δ_H 7.43) with C-3' and C-5' (δ_C 128.6), C-4' (δ_C 127.8), C-1' (δ_C 133.3), and C-5 (δ_C 114.5), and H-6 (δ_H 7.44) with C-2 (δ_C 158.0), C-4 (δ_C 161.9), C-5 (δ_C 114.5), and C-1' (δ_C 133.3) supported the partial structure of the substituted pyridinone ring moiety. HMBC correlations of the H-7 oxymethine doublet (δ_H 4.73) with C-4 (δ_C 161.9), C-2 (δ_C 158.0), C-3 (δ_C 111.6), C-11 (δ_C 86.3), C-9 (δ_C 36.4), C-8 (δ_C 39.8), and C-15 (δ_C 17.8) supported the partial structure of the tetrahydropyran moiety. The major difference in the rest of the ¹H NMR resonances of compound **1** and the reported² *N*-(*O*-methyl)septoriamycin A (**9**) is the replacement of the methyl doublet in the chain attached to C-11 of the tetrahydropyran ring by two diastereotopic oxymethylene hydrogens. This indicated that compound **1** was the C-17 oxygenated analogue of **9**. The COSY spectrum of compound **1** displayed cross peaks between the C-17 oxymethylene (δ_H 3.60, 3.49) and C-12 methine proton (δ_H 1.87). In the HMBC spectrum the oxymethylene protons showed cross peaks with C-11 (δ_C 86.3), C-12 (δ_C 36.5), and C-13 (δ_C 22.3), and the H-11 oxymethine doublet at δ_H 3.36 with C-7 (δ_C 81.2), C-9 (δ_C 36.4), C-12 (δ_C 36.5), C-13 (δ_C 22.3), C-16 (δ_C 12.6), and C-17 (δ_C 63.6) further confirming that compound **1** was 17-hydroxy-*N*-(*O*-methyl)septoriamycin A. (Figure 1)

The HRESIMS data established the molecular formula of compound **2** as C₂₅H₃₃NO₆. The ¹H and ¹³C NMR spectra were similar to those of **1** except for the presence of additional resonances [(δ_{H} 2.06) δ_{C} 21.1 (CH₃), δ_{C} 171.2 (CO)] originating from an *O*-acetyl group. HMBC correlations between the C-12 oxymethylene group and the acetyl carbonyl carbon indicated that compound **2** was 17-*O*-acetyl-*N*-(*O*-methyl)septoriamycin A. The remaining HMBC and COSY correlations were identical to those of compound **1**. Both ³*J*_{7,8} and ³*J*_{10,11} values were 10.2 Hz indicating that these protons are *trans* – diaxially oriented. ROESY correlations of compound **1** (Figure 1) and **2** were identical to those observed for septoriamycin A (**8**) suggesting that these two compounds had the same relative configurations. Since we have previously assigned the absolute configuration of septoriamycin A on the basis of X-ray diffraction data,² and all these compounds presumably share a common biosynthetic origin, compounds **1** and **2** also have 7*R*, 8*R*, 10*S*, 11*R*, and 12*R* absolute configuration. It is further supported by their dextrorotatory specific rotations.

The HRESIMS data of **3** established its molecular formula as C₂₃H₃₁NO₅. Comparison of the NMR spectra of **3** with those of **8** showed that the major difference was the replacement of a methyl triplet (δ_{H} 0.89) of the latter by a methyl doublet (δ_{H} 1.16) and an oxymethine doublet (δ_{H} 3.78) in the former. These changes could be attributable to the substitution of one of the C-13 diastereotopic methylene hydrogens in **8** with a hydroxy group indicating that compound **3** is the 13-hydroxy analogue of *N*-(*O*-methyl)septoriamycin A (**9**). The methyl doublet at δ_{H} 0.93 (17-CH₃) showed HMBC cross peaks with C-11 (δ_{C} 88.9), C-12 (δ_{C} 41.4), and C-13 (δ_{C} 68.9) and the oxymethine multiplet at δ_{H} 3.78 with C-11 (δ_{C} 88.9), C-12 (δ_{C} 41.4), and C-17 (δ_{C} 15.9) confirming the C-13 location of the secondary hydroxy group and, thus, compound **3** was 13-hydroxy-*N*-(*O*-methyl)septoriamycin A.

ROESY data and ¹H NMR coupling constants of compound **3** showed close correlations to those reported² for septoriamycin A (**8**). Our attempts to determine the absolute configuration at C-13 by Mosher analysis were unsuccessful. Treatment of compound **3** with the *R*- and *S*-Mosher's acid chlorides afforded a mixture not worth resolving. Methylation prior to acylation also yielded a mixture of products. The absolute configuration of compound **3** was proposed by a ³*J*_{12, 13} - based comparison of compounds **3** and **4** (*vide infra*).

Compound **4** had the same molecular formula, C₂₃H₃₁NO₅, as that of **3** based on HRESIMS data. The ¹H NMR spectra of these compounds were similar except for the down-field shift of the oxymethine proton (δ_{H} 4.20) and the up-field shift of a methyl doublet (δ_{H} 1.00). The COSY and HMBC spectra of compound **4** showed the same correlations as those observed for compound **3** suggesting that both compounds have the same gross structure. Identical coupling constants and ROESY correlations for the protons in the tetrahydrofuran moiety indicated that they had the same relative configuration except at C-13. Since compounds **3**, **4**, and **8** presumably share a common biosynthetic origin, compounds **3** and **4** also have 7*R*, 8*R*, 10*S*, 11*R*, 12*R* absolute configuration. As described earlier for compound **3**, our attempts to determine the absolute configuration of the C-13 stereogenic center by Mosher analysis were unsuccessful. Thus, we used a *J*-based approach relying on ¹H NMR coupling constants combined with ROESY correlations, to assign the C-13 absolute configuration of compounds **3** and **4**. An observed ³*J*_{12, 13} value of 7.2 Hz of **3**, suggested a dihedral angle of ca 30° or 150° between H-12 and H-13. Eight rotamers are possible for **3** with these dihedral angles for the *S* (**3a** – **3d**) and *R* (**3e** – **3h**) epimers (Figure 2). Observed ROESY correlations between H-12 and H-13, H-13 and CH₃-17, and CH₃-14 – CH₃-17 ruled out all conformers except **3a** as the probable most abundant rotamer for compound **3** in solution indicating a 13*S* absolute configuration. Similarly, an observed ³*J*_{12, 13} of 1.0 Hz for **4** supported the fact that the dihedral angle between H-12 and H-13 was 90° as shown in

Figure 2 for the *13S* (**4a** – **4b**) and *R* (**4c** – **4d**) epimers. In the ROESY spectrum, H-12 showed correlation with H-13 and CH₃-14 and absence of interaction between CH₃-14 and CH₃-17 indicating **4a** as the dominant rotamer and, hence, *13R* as absolute configuration.

Methylation of compounds **3** and **4** with diazomethane afforded several products. Treatment of septoriamycin A (**8**) with diazomethane as a model gave three products, whereas methylation with MeI and Cs₂CO₃ afforded a single compound which was identified as analogue **9**. The products of diazomethane methylation of septoriamycin A were separated by chromatography. All these products had the same molecular formula, C₂₄H₃₃NO₄, by HRESIMS, suggesting that they were di-*O*-methyl derivatives. The ¹H NMR spectrum of the least polar compound (**10a**) was similar to that of **9** except for the presence of an additional *O*-methyl resonance in the former. The ¹³C NMR spectrum of this compound showed changes in resonances in the pyridone ring. A down-field shift of C-4 (δ_C 177) indicated that the carbonyl group now resided at this carbon suggesting **10a** was *N*,2-di-*O*-methylseptoriamycin A. A hypsochromic shift of the UV absorption (λ_{\max} 278.9 nm) when compared to **9** (λ_{\max} 295 nm)¹¹ and COSY and HMBC correlations further supported this structure. The ¹H and ¹³C NMR spectra of compound **10b** indicated that it existed as a 3:1 mixture of two rotamers about the C-3 – C-7 bond. Their ¹H and ¹³C NMR spectra were similar to those of compound **9** but had an additional methoxy resonance. HMBC correlations showed that the additional methoxy group correlated to C-4 indicating that **10b** was *N*,4-di-(*O*-methyl)septoriamycin A. At 100 °C in pyridine, duplicated ¹H NMR resonances coalesced but were restored to the original ratio when the temperature returned to ambient conditions. Atropisomers of 3-cyclohexyl- or 3-cycloheptyl-*N*,4-dihydroxy-2-pyridone alkaloids have previously been isolated from several fungal species.^{11–15} Even though a number of 3-pyrano-*N*,4-dihydroxy-2-pyridone alkaloids similar to septoriamycin A analogues have been identified¹⁶ from fungal sources, none have been reported to exist as rotamers. The introduction of a 4-methoxy group appears to cause restricted rotation about the C-3-C-7 bond giving rise to rotamers. The most polar compound (**10c**) showed ionic characteristics. ¹H and ¹³C NMR data indicated that the compound also existed as a 3:1 mixture of two diastereomeric rotamers. HMBC correlations showed that the methoxy resonance correlated to C-2 and C-4 suggesting that **10c** is the 2,4-di-*O*-methylpyridinone-*N*-oxide analogue of septoriamycin A. The presence of the 4-methoxy group appears to cause restricted rotation about the C-3-C-7 bond. At 100 °C in pyridine duplicated ¹H NMR resonances coalesced. Even though the original ratio of rotamers reappeared at ambient temperature some decomposition of **10c** was also observed.

The known perylenequinones, (+)-cercosporin (**5**),⁴ (+)-14-*O*-acetylcercosporin (**6**),^{4,5} and (+)-di-*O*-acetylcercosporin (**7**),⁵ lumichrome,⁹ and brassicasterol,¹⁰ were identified by comparing their spectroscopic data with literature data. The absolute configurations of **5–7** were determined by comparing experimental and reported electronic circular dichroism data.^{17–18} The helicity of compounds **5–7** was confirmed as *M* (*aS*) and the absolute configuration of both C-14 and C-17 as *R*.¹⁹

Compounds **1–4** showed no antimicrobial, antifungal, antiprotozoal, phytotoxic, or cytotoxic activity in vitro. Compounds **5–7** showed moderate in vitro antiplasmodial activity (Table 3) but were cytotoxic to Vero cells. Their low selectivity indices (ratio of cytotoxicity vs. antiplasmodium activity) preclude them as antimalarial drug leads. Even though the plant pathogen *S. pistaciarum* is host-specific to pistachio, compounds **5–7** showed nonspecific moderate phytotoxic activity towards both bentgrass (*A. stolonifera*) and lettuce (*L. sativa* cv. L., Iceberg) in the presence of light (Table 4). General phytotoxicity of phytotoxins from host-specific pathogens is very common. Biosynthesis of cercosporin (**5**) appeared to be controlled by numerous environmental and physiological factors and their production has been linked to the pathogenicity of fungi.^{6,7} The possible mechanism of phytotoxic activity

of this type of compounds has previously been attributed to their ability to generate reactive oxygen species in the presence of light.⁶ This suggested that the selective inhibition of the plant-like metabolic pathways in the apicoplast of malaria parasite¹ is not responsible for the observed antimalarial activity of compounds **5–7**. Septoriamycin A (**8**), with demonstrated antiplasmodial and antifungal activities,² exhibited significant antileishmanial activity with an IC₅₀ of 0.11 μM and an IC₉₀ of 0.29 μM (Table 5) and was more potent than the positive controls pentamidine and amphotericin B. Compounds **5–7** also showed significant antileishmanial activity with IC₅₀ values of 1.14 μM, 1.7 μM, and 3.1 μM, respectively (Table 5).

Compound **5** was moderately active against both methicillin-sensitive and methicillin-resistant *Staph. aureus* with MIC values 2.5 and 5.0 Ng/mL (4.7 and 9.4 NM), respectively. In the same assay, the positive control, ciprofloxacin had a MIC value of 0.37 Ng/mL (1.11 NM) against both methicillin-sensitive and methicillin-resistant *Staph. aureus*.

The cytotoxic potential of compounds **5–7** was further evaluated against a panel of human solid tumor cell lines (SK-MEL, KB, BT-549, SK-OV-3) and pig kidney epithelial cells (LLC-PK₁₁) (Table 6). Moderate cytotoxicity was observed against all the cell lines. The di-*O*-methyl derivatives of septoriamycin A, **10a**, **10b**, and **10c** were also evaluated for the above activities and compound **10c** exhibited weak antiplasmodial activity (Table 3).

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were obtained using a Rudolph Research Analytical Autopol IV automatic polarimeter model 589-546. Melting points were measured with a Uni-melt, Thomas Hoover capillary melting point apparatus. UV and IR spectra were determined on a Varian-50 Bio UV visible spectrophotometer and a Bruker-Tensor-27 infrared spectrophotometer, respectively. NMR spectra were recorded on a Varian-Mercury-plus-400 or Varian Unity-Inova-600 spectrometer using CDCl₃ and methanol-*d*₄ unless otherwise stated. MS data were obtained from an Agilent Series 1100 SL equipped with an ESI source (Agilent Technologies, Palo Alto, CA, USA). Column chromatography and preparative TLC were carried out using Merck silica gel 60 (230–400 mesh) and silica gel GF plates (20 × 20 cm, thickness 0.25 mm), respectively. HPLC analysis was conducted on an Hewlett-Packard Agilent 1100 with diode array detector.

Fermentation, Extraction, and Isolation

The plant pathogenic fungus, *Septoria pistaciarum* Caracc. (ATCC 22201) was obtained from the American Type Culture Collection, Manassas, VA and it was grown as previously reported.²

The oily extract (3.3 g) was chromatographed over Sephadex LH-20 and eluted with 80% MeOH in CHCl₃ to give 16 fractions. Fractions 2–11 which showed antiplasmodial activity and were combined and chromatographed over a reversed phase C₁₈ column eluting with a gradient of 10 to 100% MeOH-H₂O to yield eight fractions. Subfractions 2, 3, and 4 were combined and separated on Sephadex LH-20 (MeOH) to give compounds **1** (5.0 mg) and **8** (35 mg). Subfraction 5 was chromatographed over a silica gel column using CH₂Cl₂-hexanes as the eluent to give compound **2** (8.0 mg). Combined fractions 6, 7, and 8 were chromatographed over a silica gel column using CH₂Cl₂-hexanes as the eluent to give 10 subfractions. Subfraction 2, 3, and 5 were combined and further separated using a C₁₈ reversed phase HPLC column eluting with MeCN:H₂O, (1:1), flow rate 3.5 mL/min, to give compounds **3** (3.5 mg) and **4** (8.0 mg). Subfractions 6, 7, and 8 were combined and purified on Sephadex LH-20 (MeOH) to give compounds **5** (14.0 mg), **6** (12.0 mg), and lumichrome

(3.0 mg). Subfractions 9 and 10 were combined and chromatographed over a silica gel column using CH₂Cl₂-hexanes as the eluent to give compound **7** (10.0 mg) and brassicasterol (8.0 mg).

Compound 1—amorphous powder: $[\alpha]_D^{26} +112$ (*c* 0.05, MeOH); UV (MeOH); λ_{\max} (log *e*) 205.0 (3.56), 240.9 (3.51), 297.0 (2.79) nm; IR (CHCl₃) ν_{\max} 3400, 3207, 2967, 2929, 1647, 1555, 1454, 1049 cm⁻¹; ¹H and ¹³C NMR data (see Table 1); HRESIMS [M – H]⁺ *m/z* 400.2118 (calcd for [C₂₃H₃₁NO₅ – H]⁺, 400.2124).

Compound 2—amorphous powder: $[\alpha]_D^{26} +101$ (*c* 0.05, MeOH); UV (MeOH); UV (MeOH) λ_{\max} (log *e*) 204.0 (3.44), 240.0 (3.25), 295.9 (2.56) nm; IR (CHCl₃) ν_{\max} 3331, 2967, 2925, 1740, 1651, 1230, 1045 cm⁻¹; ¹H and ¹³C NMR data (see Table 1); HRESIMS [M + H]⁺ *m/z* 444.2370 (calcd for [C₂₅H₃₃NO₆ + H]⁺, 444.2386).

Compound 3—amorphous powder: $[\alpha]_D^{26} -38$ (*c* 0.43, CH₃OH); UV (MeOH); λ_{\max} (log *e*) 208.2 (3.94), 241.9 (3.92), 297.0 (3.19) nm; IR (CHCl₃) ν_{\max} 3405, 3201, 2965, 2929, 1644, 1551, 1455, 1219, 755 cm⁻¹; ¹H and ¹³C NMR data (see Table 1); HRESIMS [M + H + Na]⁺ *m/z* 425.2172 (calcd for [C₂₃H₃₁NO₅ + H + Na]⁺, 425.2178).

Compound 4—amorphous powder: $[\alpha]_D^{26} +130$ (*c* 0.2, MeOH); UV (MeOH); λ_{\max} (log *e*) 207.1 (3.01), 241.0 (2.95), 295.1 (2.10) nm; IR (CHCl₃) ν_{\max} 3422, 3159, 2926, 1641, 1541, 1456 cm⁻¹; ¹H and ¹³C NMR data (see Table 1); HRESIMS [M + H + Na]⁺ *m/z* 425.2174 (calcd for [C₂₃H₃₁NO₅ + H + Na]⁺, 425.2178).

Methylation of Septoriamycin A (**8**) with MeI

A mixture of MeI (4 mL), Cs₂CO₃ (10.0 mg), and compound **8** (10.0 mg) in acetone was stirred at room temperature for 5 h. The reaction mixture was filtered, and the solvent was evaporated. The product was dissolved in CH₂Cl₂ and passed through a plug of florisil to give *N*-(*O*-methyl)septoriamycin A (**9**).

Methylation of Septoriamycin A (**8**) with Diazomethane

A solution of **8** (60.0 mg) in MeOH was treated with excess diazomethane in Et₂O at 0 °C for 2 h. The solvent was evaporated and the mixture was separated by PTLC (40% EtOAc in hexanes) to yield compounds **10a** (8 mg), **10b**, and **10c**. Compounds **10b** and **10c** were further purified by HPLC using a reversed phase Luna C₁₈ column (1×25 cm) with MeOH: H₂O (92:8) as the mobile phase at a flow rate of 4 mL/min, to give compounds **10b** (5 mg) and **10c** (6.0 mg).

Compound 10a—amorphous powder: $[\alpha]_D^{26} +14$ (*c* 0.5, MeOH); UV (MeOH); λ_{\max} (log *e*) 205.0 (3.75), 232.0 (3.68), 278.9 (3.41) nm; IR (CHCl₃) ν_{\max} 2959, 2929, 2873, 1627, 1547, 1469, 1046 cm⁻¹; ¹H and ¹³C NMR data (see Table 2); HRESIMS [M + Na]⁺ *m/z* 422.2307 (calcd for [C₂₄H₃₃NO₄ + Na]⁺, 422.2307).

Compound 10b—amorphous powder: UV (MeOH) λ_{\max} (log *e*) 204.0 (3.39), 235.0 (3.18), 308.0 (2.65) nm; IR (CHCl₃) ν_{\max} 2958, 2929, 1740, 1656, 1524, 1457 cm⁻¹; ¹H and ¹³C NMR data (see Table 2); HRESIMS [M + H]⁺ *m/z* 400.2472 (calcd for [C₂₄H₃₃NO₄ + H]⁺, 400.2487).

Compound 10c—amorphous powder: UV (MeOH) λ_{\max} (log *e*) 207.0 (2.58), 240.9 (2.47), 307.1 (1.70) nm; IR (CHCl₃) ν_{\max} 2900, 2850, 1575, 1450 cm⁻¹; ¹H and ¹³C NMR

data (see Table 2); HRESIMS $[M + Na]^+ m/z$ 422.2279 (calcd for $[C_{24}H_{33}NO_4 + Na]^+$, 422.2307).

Compound 5—Red crystals: mp 239 °C (lit.¹⁷ 240–241.5 °C); UV (MeOH) λ_{max} (log ϵ) 210.0 (3.71), 221.0 (3.72), 267.0 (3.55), 470.0 (3.42), 563.0 (2.98) nm; IR (CHCl₃) ν_{max} 3396, 2924, 1616, 1267 cm⁻¹ ¹H and ¹³C NMR and CD data were consistent with those reported.^{4,17,19}

Compound 6—Red crystals: mp 135 °C (lit.⁵ 134 °C); UV (MeOH) λ_{max} (log ϵ) 222.0 (3.9), 269.0 (3.74), 471.0 (3.62), 563.0 (3.2) nm; IR (CHCl₃) ν_{max} 3338, 1735, 1616, 1266 cm⁻¹ ¹H and ¹³C NMR and CD data data were consistent with those reported.^{4,5}

Compound 7—Red amorphous powder: UV (MeOH) λ_{max} (log ϵ) 222.0 (3.9), 270.0 (3.84), 470.1 (4.0), 563.3 (3.26) nm; IR (CHCl₃) ν_{max} 2921, 1736, 1617, 1211 cm⁻¹ ¹H and ¹³C NMR and CD data were consistent with those reported.⁵

Antiplasmodial Assay

The antiplasmodial activity was determined against D6 (chloroquine sensitive) and W2 (chloroquine resistant) strains of *Plasmodium falciparum* in an in vitro assay as described earlier.²⁰ Artemisinin and chloroquine were included as the drug controls and IC₅₀ values were computed from the dose response curves using Microsoft Excel software.

Phytotoxicity Assay

The bioassay for phytotoxicity was carried out according to the procedure described by Dayan *et al.*²¹ using bentgrass (*Agrostis stolonifera*) and lettuce (*Lactuca sativa* cv. L., Iceberg), in 24-well plates. Test compounds (1 mg each) were dissolved in 100 μ L of acetone and a 20 μ L aliquot of each solution was pipetted onto the filter paper and dried for 30 min by airflow in a sterile biohazard hood. Water (200 μ L) was added after placing the dried and sample impregnated filter paper in the well. The solvent controls were treated identically, using the solvent described above. Phytotoxicity was ranked visually. The ranking of phytotoxic activity was based on a scale of 0 to 5 with 0 showing no effect and 5 no growth.

Antileishmanial Assay

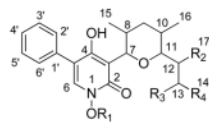
The in vitro antileishmanial activity of the compounds was carried out on a culture of *Leishmania donovani* promastigotes as described earlier.²² Pentamidine and amphotericin B were used as standard antileishmanial agents. The IC₅₀ values for each compound were computed from the growth inhibition curve using Microsoft Excel software.

Antimicrobial Assay

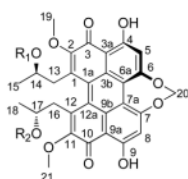
All organisms were obtained from the American Type Culture Collection (Manassas, VA) and included the fungi *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906, and the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the CLSI methods^{23,24} as described by Samoylenko *et al.*²⁵ The drug controls ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were included in each assay.

Cytotoxicity Assay for Mammalian Cells

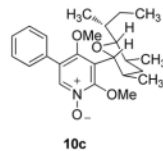
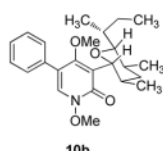
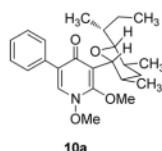
In vitro cytotoxicity was determined against a panel of mammalian cells that included kidney fibroblast (Vero), kidney epithelial (LLC-PK₁₁), malignant melanoma (SK-MEL), oral epidermal carcinoma (KB), breast ductal carcinoma (BT-549), and ovary carcinoma (SK-OV-3) cells as described earlier.²⁶ The number of viable cells was determined by using Neutral Red dye and IC₅₀ values were obtained from dose response curves. Doxorubicin was used as a positive control.



	R ₁	R ₂	R ₃	R ₄
1	CH ₃	CH ₂ OH	H	CH ₃
2	CH ₃	CH ₂ OAc	H	CH ₃
3	CH ₃	CH ₃	OH (S)	CH ₃
4	CH ₃	CH ₃	OH (R)	CH ₃
8	H	CH ₃	H	CH ₃
9	CH ₃	CH ₃	H	CH ₃



	R ₁	R ₂
5	H	H
6	OAc	H
7	OAc	OAc



Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

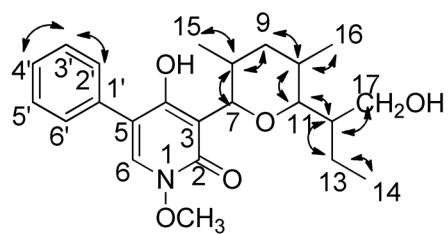
Acknowledgments

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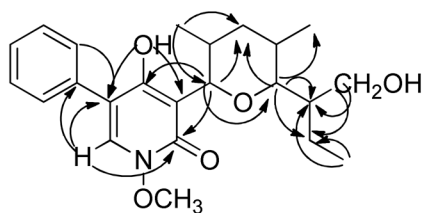
REFERENCES AND NOTES

1. Bajsa J, Singh K, Nanayakkara D, Duke SO, Rimando AM, Evidente A, Tekwani BL. *Biol Pharm Bull.* 2007; 30:1740–1744. [PubMed: 17827731]
2. Kumarihamy M, Fronczek FR, Ferreira D, Jacob M, Khan SI, Nanayakkara NPD. *J Nat Prod.* 2010; 73:1250–1253. [PubMed: 20550123]

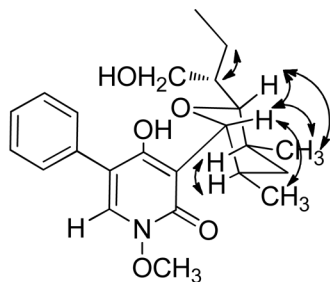
3. We did not assign a trivial name for compound **8**. This compound was recently synthesized by Fotiadou and Zografos and named septoriamycin A. Fotiadou AD, Zografos AL. *Org Lett*. 2011; 13:4592–4595. [PubMed: 21819096]
4. Tabuchi H, Tajimi A, Ichihara A. *Biosci Biotech Biochem*. 1994; 58:1956–1959.
5. Assante G, Locci R, Camarda L, Merlini L, Nasini G. *Phytochemistry*. 1997; 16:243–247.
6. Daub ME, Ehrenshaft M. *Annu Rev Phytopathol*. 2000; 38:461–490. [PubMed: 11701851]
7. You BJ, Lee MH, Chung KR. *Can J Microbiol*. 2008; 54:259–269. [PubMed: 18388998]
8. Lynch FJ, Geoghegan MJ. *Trans Br Mycol Soc*. 1979; 72:31–37.
9. Ding ZG, Zhao JY, Yang PW, Li MG, Huang R, Cuio XL, Wen ML. *Magn Reson Chem*. 2008; 47:366–370. [PubMed: 19165845]
10. Lee JW, Lee DY, Cho JG, Baek NI, Lee YH. *J Appl Biol Chem*. 2010; 53:207–211.
11. Cai P, Smith D, Cunningham B, Brown-Shimer S, Katz B, Pearce C, Venables D, Houck D. *J Nat Prod*. 1999; 62:397–399. [PubMed: 10075801]
12. Teshima Y, Shin-ya K, Shimazu A, Furihata K, Chul HS, Furihata K, Hayakawa Y, Nagai K, Seto H. *J Antibiot*. 1991; 44:685–687. [PubMed: 2071495]
13. Isaka M, Tanticharoen M, Kongsaree P, Thebtaranonth Y. *J Org Chem*. 2001; 66:4803–4808. [PubMed: 11442408]
14. Wagenaar MM, Gibson DM, Clardy. *J Org Lett*. 2002; 4:671–673.
15. De Silva ED, Geiermann AS, Mitova MI, Kuegler P, Blunt JW, Cole ALJ, Munro MHG. *J Nat Prod*. 2009; 72:477–479. [PubMed: 19323568]
16. Henning JJ, Gademann K. *Nat Prod Rep*. 2010; 27:1168–1185. [PubMed: 20532354]
17. Yamazaki S, Ogawa Tadatomu. *Agr Biol Chem*. 1972; 36:1707–1718.
18. Morgan BJ, Dey S, Johnson SW, Kozlowski MC. *J Am Chem Soc*. 2009; 131:9413–9425. [PubMed: 19489582]
19. Morgan BJ, Mulrooney CA, Kozlowski MC. *J Org Chem*. 2010; 75:44–56. [PubMed: 19894744]
20. Bharate SB, Khan SI, Yunus NAM, Chauth SK, Jacob MR, Tekwani BL, Khan IA, Singh IP. *Bioorg Med Chem*. 2007; 15:87–96. [PubMed: 17070063]
21. Dayan FE, Romagni JG, Duke SO. *J Chem Ecol*. 2000; 26:2079–2094.
22. Machumi F, Samoylenko V, Yenesew A, Derese S, Midiwo JO, Wiggers FT, Jacob MR, Tekwani BL, Khan SI, Walker LA, Muhammad I. *Nat Prod Commun*. 2010; 5:853–858. [PubMed: 20614808]
23. NCCLS. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically M7-A5. National Committee on Clinical Laboratory Standards. 2000; 20(2)
24. NCCLS. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium-Forming Filamentous Fungi; Proposed Standard, M38-P. National Committee on Clinical Laboratory Standards. 1998; 18(13)
25. Samoylenko V, Jacob MR, Khan SI, Zhao J, Tekwani BL, Midiwo JO, Walker LA, Muhammad I. *Nat Prod Commun*. 2009; 4:791–796. [PubMed: 19634324]
26. Mustafa J, Khan SI, Ma G, Walker LA, Khan IA. *Lipids*. 2004; 39:167–172. [PubMed: 15134144]



Key COSY (↔) correlations



Key HMBC (↔) correlations



Key ROESY (↔) correlations

Figure 1.
COSY, HMBC, and ROESY correlations of compound **1**.

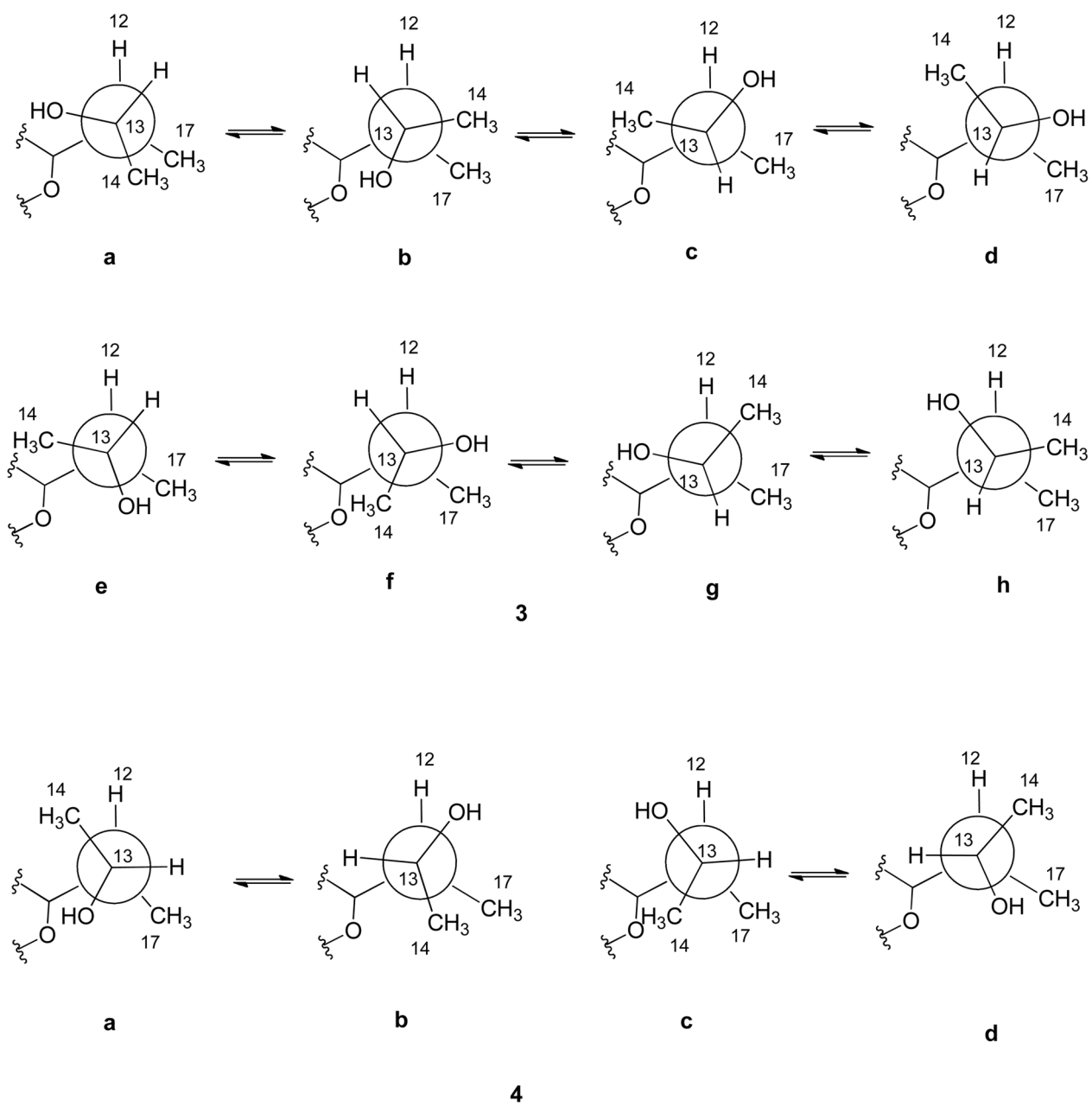


Figure 2.
Rotamer representation of compounds 3 and 4

Table 1

¹H, and ¹³C, NMR Data for Compounds 1–4 in CDCl₃/Methanol-d₄.

	1		2		3		4	
position	δ_c^b	δ_H^a (J in Hz)	δ_c^b	δ_H^a (J in Hz)	δ_c^b	δ_H^a (J in Hz)	δ_c^b	δ_H^a (J in Hz)
2	158.0		158.0		158.0		158.2	
3	111.6		111.4		112.0		112.0	
4	161.9		161.8		161.6		161.7	
5	114.5		114.3		114.5		114.8	
6	133.0	7.43, s	133.1	7.44, s	132.9	7.42, s	132.8	7.43, s
7	81.2	4.73, d (10.2)	81.3	4.74, d (10.2)	80.8	4.70, d (10.2)	80.9	4.66, d (10.2)
8	39.8	1.87, m	36.4	1.91, m	36.2	1.91, m	36.7	1.91, m
9	36.4	1.34, q (12.6)	36.5	1.30, q (12.6)	42.6	1.06, q (12.6)	42.2	1.16, q (12.0)
		1.98, brd (13.2)		2.00, (overlap)		1.80, dt (13.8, 3.6)		1.84, dt (13.2, 3.6)
10	35.8	1.69, m	36.0	1.64, m	34.1	2.03, m	32.9	1.89, m
11	86.3	3.36, d (10.2)	86.2	3.33, d (10.2)	88.9	3.14, d (10.2)	88.5	3.12, dd (10.2, 2.8)
12	36.5	1.87, m	37.0	2.00, m	41.4	2.05, m	38.7	1.75, m
13	22.3	1.02, m, 1.58, m	22.3	1.02, m, 1.55, m	68.9	3.78, m (7.2, 7.2)	66.0	4.20, m (6.6, 1.0)
14	12.6	0.88, t (7.2)	12.6	0.89, t (7.2)	23.1	1.16, d (6.6)	22.8	1.16, d (6.3)
15	17.8	0.91, d (6.6)	17.7	0.91, d (7.2)	17.9	0.85, d (6.6)	17.9	0.87, d (6.6)
16	17.1	0.95, d (6.6)	17.0	0.97, d (7.2)	17.7	0.83, d (6.6)	17.7	0.84, d (6.6)
17	63.6	3.49, dd (10.6, 3.6)	65.3	3.91, dd (11.2, 5.4)	15.9	0.93, d (7.2)	10.5	1.01, d (7.2)
		3.60, dd (10.6, 3.6)		3.98, dd (11.6, 4.5)				
1'	133.3		133.4		133.4		133.7	
2', 6'	129.2	7.44, d (7.3)	129.3	7.44, d (7.3)	129.2	7.43, d (7.3)	129.4	7.43, d (7.3)
3', 5'	128.6	7.39, t (7.2)	128.6	7.40, t (7.3)	128.5	7.38, t (7.8)	128.6	7.36, d (7.2)
4'	127.8	7.32, t (7.2)	127.9	7.34, t (7.3)	127.7	7.32, t (7.2)	127.8	7.30, t (7.2)
OH		9.63, s		9.47, s		9.63, s		9.65, s
OCH ₃	65.0	4.04, s	65.0	4.06, s	65.0	4.04, s	65.0	4.05, s
COCH ₃			21.1	2.06, s				
COCH ₃			171.2					

¹H NMR spectra recorded at 600 MHz,

¹³C NMR spectra recorded at 100 MHz

Table 2

¹H and ¹³C NMR Data Methylated Compounds **10a**, **10b**, and **10c** in CDCl₃/Methanol-*d*₄.

position	10a			10b			10c		
	δ_c^b	δ_H^a (J in Hz)	δ_c^b	δ_H^a (J in Hz)	δ_c^b	δ_H^a (J in Hz)	δ_c^b	δ_H^a (J in Hz)	δ_c^b
2	155.0	159.2	157.2				158.0	156.0	
3	119.5	124.3	122.8				125.6	125.3	
4	177.0	164.9	164.8				155.7	157.0	
5	134.6	117.2	115.9				128.5	128.5	
6	132.7	7.51, s	133.4	134.0	7.44, s	7.49, s	139.6	139.7	8.14, s
7	78.4	4.63, d (10.2)	79.4	79.5	4.56, d (10.2)	4.21, d (10.2)	79.4	80.7	4.33, d (10.4)
8	36.3	2.0, m	33.8	33.8	2.3, m	2.3, m	32.6	32.9	2.44, m
9	43.4	1.78, m	43.2	43.1	1.79, m	1.84, m	43.1	43.1	1.86, m
		1.02, m			1.04, m	1.06, m			0.96, m
10	33.7	1.69, m	32.3	31.9	1.72, m	1.72, m	32.4	32.5	1.88, m
11	89.2	2.92, d (12.0)	89.0	88.9	2.97, d (12.0)	2.87, d (12.0)	89.8	90.0	2.92, d (10.0)
12	34.2	1.54, m	36.1	36.7	1.57, m	1.57, m	36.0	36.0	1.60, m
13	22.8	1.54, m	22.6	21.9	1.53, m	1.53, m	22.5	22.5	1.5, m
		0.82, m			0.85, m	0.85, m			1.1, m
14	13.0	0.84, t (7.2)	12.7	12.4	0.84, t (6.6)	0.84, t (6.6)	12.9	12.8	0.87, t (6.6)
15	17.6	0.68, d (6.6)	17.5	17.8	0.80, d (6.6)	0.80, d (6.6)	17.6	17.6	0.81, d (6.6)
16	17.7	0.77, d (6.6)	17.3	17.6	0.75, d (6.6)	0.68, d (6.6)	17.7	17.8	0.67, d (6.6)
17	17.0	0.89, d (6.6)	16.8	17.0	0.91, d (6.6)	0.91, d (6.6)	17.2	17.2	0.90, d (6.6)
1'	126.6		133.8	133.8			133.0	133.0	
2', 6'	128.9	7.56, d (7.2)	128.6	128.5	7.43, d (7.2)	7.43, d (7.2)	129.0	128.9	7.48, d (7.5)
3', 5'	128.3	7.33, t (7.2)	128.4	128.5	7.39, t (7.2)	7.39, t (7.2)	128.9	128.7	7.42, d (7.5)
4'	127.8	7.25, t (7.2)	127.6	127.5	7.34, t (7.2)	7.34, t (7.2)	129.8	129.8	7.39, t (7.3)
OCH ₃	66.8	4.01, s	64.5	64.5	4.08, s	4.08, s	61.7	62.1	3.36, s
OCH ₃	64.4	4.0, s	61.5	61.2	4.01, s	3.34, s	60.6	60.4	4.16, s

^a¹H NMR spectra recorded at 600 MHz,^b¹³C NMR spectra recorded at 100 MHz

Table 3

Antiplasmodial Activity of Compounds **5–7** and **10c**

Compound	Chloroquine-sensitive (D6)-clone	Chloroquine-resistant (W2)-clone	Cytotoxicity to Vero cells
	IC ₅₀ μ M	S.I.	IC ₅₀ μ M
5	1.08	4.8	1.62
6	2.78	1.9	3.12
7	2.75	1.6	1.94
10c	6.76	>1.1	6.51
chloroquine ^a	0.03		0.31
artemisinin ^a	0.02		0.01

^aPositive controls

NC not cytotoxic

S. I. (selectivity index) = IC₅₀ for cytotoxicity/IC₅₀ for antiplasmodial activityIC₅₀: concentration causing 50% growth inhibition

Table 4Phytotoxic Activity of Compounds **5–7**

Compound	Concentration (mM)	lettuce	bentgrass
5	1.87	3	2
6	1.73	2	4
7	1.62	3	4

Concentration (mM) = 1 mg/mL

Ranking based on scale of 0 to 5

0 = no effect

5 = no growth

Table 5Antileishmanial Activity of Compounds **5–8**

Compound	IC ₅₀ μ M	IC ₉₀ μ M
5	1.14	2.81
6	1.7	8.5
7	3.1	9.7
8	0.11	0.29
pentamidine^a	2.9	5.58
amphotericin B^a	0.18	0.38

^aPositive controlsIC₅₀ concentration causing 50% growth inhibitionIC₉₀ concentration causing 90% growth inhibition

Table 6

Cytotoxic Activity [IC₅₀ (μM)] of Compounds 5–7

	SK-MEL	KB	BT-549	SK-OV-3	LLC-PK ₁₁
5	3.6	7.1	10.8	3.7	3.9
6	3.8	8.5	8.6	4.0	10.1
7	4.9	8.7	8.7	4.8	9.4
doxorubicin^a	1.6	2.6	2.6	1.5	1.6

^aPositive control.IC₅₀ = concentration causing 50% growth inhibition

SK-MEL = human malignant melanoma

KB = human epidermal carcinoma

BT-549 = human breast carcinoma (ductal)

SK-OV-3 = human ovary carcinoma

LLC-PK₁₁ = pig kidney epithelial